AR201-13831A

HPV Test Plan and Screening Level Assessment

for

Nonanoic acid, sulfophenyl ester, sodium salt
CAS #: 91125-43-8

Prepared for the HPV Challenge Program by:
The Procter & Gamble Company

December 21, 2001

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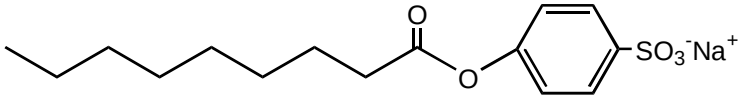
[1] Executive Summary

[1.1] CAS RN: 91125-43-8

[1.2] Substance Name: Nonanoic acid, sulfophenyl ester, sodium salt (Nonanoyloxybenzene sulfonate—NOBS)

[1.3] Structure and Synthesis

(C15 H21 O5 S Na):



NOBS can be made by reacting Nonanoyl chloride (CAS RN 764-85-2) and Sodium phenol sulfonate (CAS RN 1300-51-2). The alkyl chain of NOBS is generally linear and saturated. As the structure diagram shows the sulfonate group is predominately in the para- position. Purity of commercial production ranges from 90-99%.

[1.4] Production Volume

Nonanoic acid, sulfophenyl ester, sodium salt (Nonanoyloxybenzene sulfonate, NOBS) is a proprietary material of The Procter & Gamble Company (P&G). Total tonnage reported in the 1998 IUR was 11,100 metric tons. (1 metric ton = 2204.6 lbs).

[1.5] Use Pattern and Function

The sole use of NOBS is in P&G laundry granular and tablet detergent products intended for household use at a maximum level of 6%. Less than 20% of US granular/tablet laundry detergents contain this ingredient. NOBS is produced as an extrudate and then formulated with other detergent ingredients. P&G has marketed laundry detergent products containing NOBS in the United States for 15 years. Prior to, and since introducing NOBS to the marketplace, P&G has conducted a broad range of hazard and exposure evaluations, both alone and as used in laundry detergent products. In addition, it is estimated that NOBS-containing detergents have been used by more than 100 million consumers in washing more than 12 billion loads of laundry. P&G's extensive post-marketing surveillance and the long history of marketplace experience support the safety profile described in the human and environmental assessments.

NOBS is one of several *n*-alkanoyloxybenzene sulfonates (AOBS) that have been developed as bleach activators for inclusion in laundry detergents to create an oxygen based bleaching system. The materials were developed in response to the need for effective warm water (40-60ºC) bleaching agents to replace direct precursors of hydrogen peroxide that exhibit optimum effectiveness at close to boil temperatures (80-90ºC).

Detergent formulations containing NOBS are designed to help ensure rapid conversion of NOBS to the peroxy compound in the wash water. The peroxy compound is short-lived due to rapid bleaching activity. To be effective in the wash cycle, the peracid precursor must be highly water-soluble, undergo rapid and total perhydrolysis, and produce a peracid bleach with surface activity.

[1.6] Environmental Screening Level Assessment

NOBS is highly water soluble and non-volatile. It is degraded (>99%) during the laundry wash process, and any residual NOBS is then rapidly and completely biodegraded and highly removed during wastewater treatment (> 95% removal). With a log Kow of –0.6, NOBS bioaccumulation potential is extremely low. The environmental fate of NOBS during the main phases of its life-cycle (manufacturing, processing, consumer use) was modeled using E-FAST, a U.S. EPA screening level model. Predicted environmental concentrations (PEC) in surface water range from 0.003 ng /l to 16 μg /l, depending upon the scenario assessed. Environmental monitoring studies have not been performed, as modeled estimates suffice for this material.

Three laboratory acute ecotoxicity studies are available for NOBS – for algae, daphnia, and fish (bluegill). The lowest acute toxicity value is 9.3 mg/l for the algae, *Selenastrum capricornutum* (E*b*C50). EbC50 is the concentration of the test substance that results in a 50% reduction in algal biomass following exposure for a determined period of time, typically 72 or 96 hours. The US EPA recommended assessment factor when acute toxicity test data are available for three different aquatic taxa is 100, in order to account for various uncertainties in the measured data; therefore, the Predicted No Effect Concentration (PNEC) is 93 μg/l based on the available data.

The risk to the aquatic environment is characterized by comparing the Predicted Exposure Concentration (PEC) to the Predicted No Effect Concentration (PNEC). If the concentration in the surface water is less than the no effect concentration, then the potential for adverse effects is low. Integrating all the information currently available, the modeled NOBS PEC at manufacturing and processing sites and in surface waters following consumer use (0.003 ng/l to 16 μg/l) does not exceed the PNEC (93 μg/l). The risk characterization ratios (PEC/PNEC) range from 4.3×10^{-7} to 0.17, where the high-end prediction conservatively assumes that neither hydrolysis nor perhydrolysis occurs following discharge at the manufacturing and processing sites and removal in wastewater treatment is 95% vs 99+% observed in studies. These ratios below 1.0 confirm that the potential for adverse environmental effects from NOBS is very low. As detailed in Section [2.3], degradation products from NOBS are also predicted to have low toxicity, to be removed during wastewater treatment and are not likely to persist in the environment.

[1.7] Human Health Screening Level Assessment

An extensive database of toxicology studies exists on NOBS (alkyl chain C9) and closely related n-alkanoyloxybenzene sulfonates, differing only in carbon chain length including C8, and also a 50:50 mixture of C8 and C10. These studies include both SIDS and beyond-SIDS endpoints, and collectively demonstrate that this material possesses a low order of

toxicity. Acute toxicity studies show that NOBS is not measurably toxic by the oral or dermal routes. Studies indicate this material can be moderately to severely irritating to eyes depending on the concentration of the solution and exposure conditions. Skin irritation was slight or negligible after a four-hour exposure in a variety of dermal studies depending on concentrations tested. Longer or repeated dermal exposures to NOBS may result in increased skin irritation. An assessment of the *in vitro* and *in vivo* genotoxicity potential of NOBS shows no evidence of mutagenic or clastogenic activity. Exposure of dams to doses up to 1,500 mg/kg/day of the bleach activator during pregnancy did not result in embryotoxicity or teratogenic effects in offspring. Similarly, no adverse effects were detected on fertility parameters or reproduction following repeated exposure of up to 1,000 mg/kg/day in males and females prior to mating and during pregnancy.

The potential of NOBS to induce and elicit a skin sensitization response has been investigated using a variety of non-clinical and clinical approaches. Two different animal laboratory methodologies were used, the modified Buehler method using guinea pigs and the local lymph node assay using mice. The four animal studies included in this summary show that NOBS as a raw material has the potential to act as a weak skin sensitizer in the guinea pig although these effects are not consistently replicated in all animal studies. Clinical studies show NOBS is not a skin sensitizer in humans at the concentrations associated with use in product. Numerous studies, which included a total of over 2,000 volunteers who gave informed consent, have been conducted on NOBS and/or laundry detergents containing NOBS, demonstrating that this material can be used in product without the risk of inducing or eliciting skin sensitization reactions in humans. The weight of evidence conclusion that NOBS can be safely used in laundry products at concentrations up to 6% is also supported by peer review. In 1986, both P&G's approach to assessing skin safety for laundry products and also the use of NOBS in laundry detergents were reviewed and approved by a group of 16 international dermatologist experts.

The potential for systemic toxicity and functional alterations resulting from repeated exposure to NOBS was evaluated in subchronic toxicity studies by oral and dermal exposure routes. The results from these studies establish that repeated exposure to NOBS via the oral or dermal route does not result in systemic toxicity or histopathological changes in any of the organs or tissues examined. Studies on the absorption, distribution and excretion of radiolabeled NOBS showed the material was very poorly absorbed upon dermal exposure and rapidly absorbed and eliminated within 72 hours following oral gavage, with no evidence of accumulation in any tissue or organ.

In summary, the toxicological profile of NOBS indicates that the material has a low order of toxicity, based on a variety of acute and sub-chronic studies. The only areas where the potential for adverse effects have been observed have been slight to moderate eye and skin irritation when tested at higher concentrations and/or in repeated exposures, and potential skin sensitization, but only in animal (guinea pig) studies, not in humans.

Exposure Data - Based on the chemistry, rapid perhydrolysis during wash (>99% in 3 minutes), and removability of NOBS during wastewater treatment (>95% of the 1% remaining), there is negligible consumer exposure to this material under recommended use situations. This assessment is based on a thorough attempt to identify the intended and

reasonably foreseeable uses for laundry products containing this material, to assess those resultant exposures, and to evaluate the relevant exposures by way of a high-end analysis. A discussion of this analysis, the methods used for the exposure assessment, and their accuracy and relevance are presented. Where appropriate from a scientific standpoint, related individual exposures are aggregated thus yielding a maximum probable exposure for the chemical.

NOBS is used in household laundry detergents as a bleach activator that results in generation of an oxygen based bleaching system. Based on this use, workers and consumers may be exposed to NOBS although the type of relevant exposure varies for these two populations.

Worker Exposure - For workers, inhalation and dermal exposure to NOBS during the production, formulation or transportation process is limited due to process design that produces a low vapor pressure, non-respirable extrudate (particles of 500 - 1000 μm) as well as industrial hygiene standards and personal protective equipment that are standardly utilitized in production facilities. Employee exposure is minimized through engineering controls and good industrial hygiene practices to ensure exposure is below an OEG of 0.1 mg/m^3. Processing experience with a variety of ingredients in the manufacturing of laundry detergents confirms that these practices are effective in minimizing worker exposure. Worker exposure in commercial and industrial laundries is not expected since these are not intended uses.

Consumer Exposure - Due to the rapid perhydrolysis reaction of the material when formulated in finished product and under use conditions, the potential for consumer exposure to NOBS is very limited. Consumer monitoring studies have not been performed, as modeled estimates suffice for this material. The most relevant and anticipated exposure to consumers is via dermal exposure. Dermal exposure can result from hand laundering of fabric or using a concentrated paste for pretreatment of fabric. Exposure from hand laundering is estimated at 7.5×10^{-6} g/kg/day and exposure from fabric pretreatment is estimated at 4.1×10^{-4} g/kg/day. For these dermal exposures, only 1% is expected to be absorbed based on ADME data. Incidental exposure to unreacted NOBS can occur while scooping the product from the box or from direct exposure to the dry laundry detergent during a spill but both of these exposures would be insignificant by orders of magnitude in comparison to hand laundering or fabric pretreatment. Any residual amount of detergent that may remain on fabric after laundering would not contain NOBS due to the complete perhydrolysis of the material in the wash water. Consumer exposure to the tablet form of the product is expected to be the same as or less than with the granular form.

There is no anticipated oral exposure under recommended use conditions. Due to the complete perhydrolysis, degradation of NOBS and it's removal during waste water treatment, the potential level in drinking water is negligible to nil. Exposure calculations based on estimates of NOBS in drinking water using the EPA's E-FAST model (conservatively assumes perhydrolysis is not complete, some NOBS remains and none is removed in drinking water treatment facilities) resulted in estimated values of 3.9×10^{-8} mg/kg/day. E-FAST provides screening level estimates of concentrations of chemicals

released to the environment from consumer products and is designed to provide high end to bounding estimates of exposure as is appropriate for screening level assessments.

Consumer inhalation exposure during use is limited by a number of factors: the low vapor pressure of NOBS, its production in extrudate form, and the overall design of the laundry product as a non-friable, dense granular or tablet material. Thus, there is very little dust involved in transferring the product from the package to the washing machine so the potential for inhalation exposure from this action is negligible.

Children's Exposure - Exposure of children to NOBS and detergents containing NOBS is expected to be infrequent and negligible based on the recommended use of the product. The product is intended for use by adults and perhaps adolescents in conjunction with automatic washing machines. There may be accidental ingestion of laundry detergents containing NOBS by children however these would be infrequent and result in mild transient symptoms, if any are present, such as nausea, vomiting and/or diarrhea, consistent with the effects observed following accidental ingestion of other laundry products. Any dermal exposures by children should be limited to detergent residues on clothing, which, as discussed above, will not contain any NOBS based on the chemistry and complete perhydrolysis of the material in the wash water.

Summary of human health assessment - The data summarized above demonstrate that NOBS has a favorable safety profile for use in consumer laundry detergents. The risk to human health is characterized by comparing the estimated human exposure to the No Observed Effect Level (NOEL) from animal studies. The amount by which the NOEL exceeds the estimated exposure is referred to as the margin of exposure and this should be sufficiently large to account for several sources of uncertainty and variability in extrapolating data from animal studies to man. Based on the data presented, no adverse effects for humans are expected via any relevant exposure route. The aggregate dermal exposure from hand laundering and pretreatment of fabrics results in an estimated exposure of 4.2×10^{-4} g/kg/day. In comparing this conservative estimate to the results from the dermal subchronic study where the no effect level (NOEL) is greater than 0.4 g/kg/day, the margin of exposure is acceptable. Even at the highest dose tested in this study (2 ml/kg of a 20% test material solution) there were no systemic effects. The only effect noted was irritation at the site of application, which was dose related and limited the amount that could be repeatedly administered. Studies evaluating the dermal absorption of NOBS showed this material is very poorly absorbed through the skin—less than 1%. For potential oral exposure, if one assumes conservatively that perhydrolysis does not occur, that NOBS would be present in drinking water and not removed in drinking water treatment facilities, the calculated exposure using EFAST would be 3.9×10^{-8} mg/kg/day. The NOEL in an oral dietary study was 1.1 g/kg/day. Comparing the estimated oral exposure to the oral NOEL results in a margin of exposure of many orders of magnitude, even after accommodating inter- and intraspecies variation.

[1.8] HPV Test Plan Status

	Data Available	Data Acceptable	Testing Required
Physical/Chemical Characteristics			
Melting Point	Y	Y	N
Boiling Point	Y	Y	N
Vapor Pressure	Y	Y	N
Partition Coefficient	Y	Y	N
Water Solubility	Y	Y	N
ENVIRONMENTAL FATE			
Photodegradation	N	Not relevant	N
Stability in Water	Y	Y	N
Transport (Fugacity)	Y	Y	N
Biodegradation	Y	Y	N
Perhydrolysis	Y	Y	N
Ultimate removability	Y	Y	N
ECOTOXICITY			
Acute Toxicity to Fish	Y	Y	N
Acute Toxicity to Invertebrates	Y	Y	N
Acute Toxicity to Aquatic Plants	Y	Y	N
MAMMALIAN TOXICITY			
Acute Toxicity	Y	Y	N
Genetic Toxicity-Ames	Y	Y	N
Genetic Toxicity-Chromosomal Ab.	Y	Y	N
Unscheduled DNA Synthesis	Y	Y	N
Repeated Dose Toxicity	Y	Y	N
Developmental Toxicity	Y	Y	N
Reproductive Toxicity	Y	Y	N
Eye Irritation	Y	Y	N
Skin Irritation	Y	Y	N
Dermal Sensitization	Y	Y	N
ADME	Y	Y	N

[1.9] Sponsor's Conclusions and Recommendation

The available data on NOBS hazard and exposure demonstrates that there is negligible likelihood of harm to man and the environment during manufacture and use of this material in laundry detergents. All SIDS and other relevant endpoints are complete with reliable data, showing that the material possesses a low order of toxicity. NOBS is a P&G proprietary material and hence the production volume and use pattern are under strict control. Aquatic PEC/PNEC ratios range from 4.3×10^{-7} to 0.17. Exposure to NOBS in the workplace is limited due to a process design that produces a low vapor pressure, non-respirable extrudate. Employee exposure is further minimized through engineering controls and good industrial hygiene practices to ensure exposure is below an OEG of 0.1 mg/m^3. Consumer evaluations indicate that margins of exposure are acceptable and calculations supporting these estimates are conservative. Considering the completeness, accuracy, and relevance of both the hazard and exposure evaluations, NOBS is recommended as sufficiently studied and a low priority for further work.

[2] Environmental Assessment

[2.1] Introduction

NOBS is a proprietary bleach activator exclusively used in granular and tablet laundry detergents intended for household use. For the present assessment, the total tonnage of NOBS is thus assumed to be released down-the-drain. Each of the reports obtained was reviewed to determine adequacy according to U.S. EPA criteria and reliability per Klimisch *et al.* (1997). Robust summaries were prepared for each report with the highest Klimisch scores according to the guidelines recommended by the U.S. EPA (U.S. EPA, 1999) for each study type. These methods include consideration of the reliability, relevance and adequacy of the data in evaluating their usefulness for hazard assessment purposes. The codification described by Klimisch specifies four categories of reliability for describing data adequacy. These are:

> 1—Reliable without Restriction: Includes studies or data complying with Good Laboratory Practice (GLP) procedures, or with valid and/or internationally accepted testing guidelines, or in which the test parameters are documented and comparable to these guidelines.
> 2—Reliable with Restrictions: Includes studies or data in which test parameters are documented but vary slightly from testing guidelines.
> 3—Not Reliable: Includes studies or data in which there are interferences, or that use non-relevant organisms or exposure routes, or which were carried out using unacceptable methods, or where documentation is insufficient.
> 4—Not Assignable: Includes studies or data in which insufficient detail is reported to assign a rating, e.g., listed in abstracts or secondary literature.

Robust study summaries for endpoints with available and reliable data for NOBS are provided in Appendix A and are summarized in Tables 1 to 3.

Table 1: Physical/Chemical Property Data

PHYSICALCHEMICAL	RESULTS	Unit	PROTOCOL
Melting Point [a]	> 360	°C	Metal block method, EEC Directive 67/548. Klimisch 1
Boiling Point [a]	> 360	°C	Metal block method, EEC Directive 67/548. Not tested
MW	336	(g/mol)	
Relative Density	$D^{20}_4 = 1.236$		Pycnometer method, EEC Directive 67/548. Klimisch 1
Vapor Pressure	1.71×10^{-7}	Pa at 25°C	Vacuum micro-balance method, EEC Directive 67/548. Klimisch 1
Partition Coefficient	- 0.572	Log P_{ow}	HPLC, EEC Directive 67/548. Klimisch 1
Water Solubility	245 ± 8	g/l at 20 °C	Flask stirring method, EEC Directive 67/548. Klimisch 1
Particle size	500 - 1000	µm	Dry Sieve Analysis, CIPAC 1995 Klimisch 1

[a] Melting and boiling point estimates are irrelevant—NOBS decomposes before it melts

Table 2: Environmental fate and pathway data

ENVIRONMENTAL FATE AND PATHWAY	RESULTS	PROTOCOL
Hydrolysis	27 % at pH 6.4 at 20°C after 192 h	FI/MS/MS; "Official Journal of the European Communities" (N°L383A - A6/Water solubility). Klimisch 1
Transport and Distribution between Environmental Compartments	Air: 2.5 x 10^{-18} % Water: 99.9% Sediment: 0.13% Soil: 3 x 10^{-10} %	Calculated Fugacity Level III Type (local exposure, EQC model, Mackay et al, 1996) Klimisch 2
Perhydrolysis	> 99% degradation in the wash after 3 minutes	HPLC; "Official Journal of the European Communities" (N°L383A - A6/Water solubility)
Biodegradation	Theoretical CO_2: 87% after 28 days	Ready biodegradability, OECD 301B. Klimisch 1
Ultimate removability	99.7 ± 2.0 % removal	SCAS test, OECD 302A. Klimisch 1
Photodegradation	Not completed.	Study not relevant— material has low volatility, is degraded in the wash; residual rapidly and completely biodegraded and highly removed during wastewater treatment

Table 3: Environmental toxicity data

ECOTOXICITY	SPECIES	RESULTS	PROTOCOL
Acute Toxicity to Fish	*Lepomis macrochirus* (bluegill)	LC_{50} (96 hr) = 32 mg/l	EPA-660/3-75-009. Klimisch 2
Toxicity to Aquatic Plants (Algae)	*Selenastrum capricornutum*	72 h EbC50 = 9.3 mg/l 72 h ErC50 = 26 mg/l	OECD Guideline 201. Klimisch 1
Acute Toxicity to Aquatic Invertebrates	*Daphnia magna*	48 h EC50 > 1000 mg/l	EPA-660/3-75-009. Klimisch 2

[2.2] Fugacity modeling

Fugacity modeling was performed to estimate transport and distribution into environmental compartments. Given the very low volatility of NOBS, which implies it will not partition to the atmospheric compartment, the question of atmospheric photodegradation is not relevant from a hazard assessment standpoint. NOBS is a highly water soluble and non-volatile chemical. As a consequence, the main environmental compartment to be exposed to NOBS is the aquatic one, as shown by the fugacity model results. The EQC Model (version 1.0.1; Mackay *et a*l.,1996) was used with the chemical input parameters shown in Table 1 and 100% of NOBS released to water in order to model adequately its actual use. NOBS is readily biodegradable. US EPA recommends using half-lives of 5 days for water and soil and 20 days for sediment when predicting the environmental fate of readily biodegradable chemicals (http://www.epa.gov/opptintr/exposure/docs/halflife.htm#eqc). EQC model results are shown in Table 2. EQC predicted that 99.9% of NOBS released to the

environment is distributed to surface waters. Therefore, surface waters should be addressed in risk assessment as the relevant environmental repository.

[2.3] Environmental Fate

NOBS is degraded during the wash process, any residual NOBS is then rapidly and completely biodegraded and highly removed during wastewater treatment (Table 2). Thus, release to the aquatic environment is minimal and no additional studies are suggested for environmental fate endpoints (e.g. Photodegradation). Considering (1) the limited fraction of NOBS released to the environment after use (see below [2.3.3], degradation in the wash solution), and (2) the very low log Pow (Table 1), it is expected that the chemical will not bioaccumulate or be transferred to higher trophic levels or humans via the food chain.

Environmental safety profile of NOBS degradation products

The major degradation products of NOBS (perhydrolysis - major pathway, Appendix B) in the wash solution are pernonanoic acid and phenol sulfonate. Their environmental fate and toxicity profiles are summarized below.

Environmental fate of NOBS degradation products According QSAR (SRC Biowin v3.67) output, probability of linear biodegradability of pernonanoic acid is 0.77; non linear biodegradability probability is 0.92. Probability of linear biodegradability of phenol sulfonate is 0.56; non linear biodegradability probability is 0.60. These high probabilities of biodegradation indicate that the major degradation products of NOBS will also be removed during wastewater treatment and are not likely to persist in the environment.

Environmental toxicity of NOBS degradation products According to QSAR (ECOSAR v0.99e) output, the predicted aquatic toxicity of pernonanoic acid and phenol sulfonate are:

Pernonanoic acid
SMILES : CCCCCCCCC(=O)OO. MOL FOR: C9 H18 O3. MOL WT : 174

ECOSAR Class	Organism	Duration	End Pt	mg/L
Neutral Organic	Fish	14-day	LC50	1738
Peroxy Acids	Fish	96-hr	LC50	0.21
Peroxy Acids	Daphnid	48-hr	LC50	18

Phenol sulfonate
SMILES : Oc1(ccc(S(=O)(=O)(O))cc1). MOL FOR: C6 H6 O4 S1. MOL WT : 174

ECOSAR Class	Organism	Duration	End Pt	mg/L
Neutral Organic SAR	Fish	14-day	LC50	1738
Phenols-acid	Fish	96-hr	LC50	1057
Phenols-acid	Daphnid	48-hr	LC50	240
Phenols-acid	Green Algae	96-hr	EC50	6637

According to QSAR modeling, phenol sulfonate toxicity is low and its contribution to the overall environmental risk is negligible. In contrast, one predicted LC50 value of peracids is low. However, over 90% of the pernonanoic acid bleach is consumed during the first 8 minutes of the wash cycle (based on consumer data of average soil load in a wash and timed trials). Moreover, any pernonanoic acid that survives the wash will react with components in wastewater. The resulting carboxylate will degrade very quickly. Therefore, the environmental safety profile of NOBS degradation products formed during the wash is judged to be favorable.

[2.3.1] Predicted removal of NOBS in waste water treatment plants

Considering the rapid and complete biodegradability and high removal (> 99%) in a semi-continuous activated sludge test, removal in waste water treatment plants is predicted to be greater than 95%. This applies to the less than 1% fraction of NOBS released to municipal wastewater handling and treatment following consumer use and destruction of NOBS during washing (see degradation of NOBS, Appendix B).

[2.3.2] Ecosystem Exposures Related to Emissions from the Production, Handling, or Formulation of the Chemical in Industrial Facilities

The NOBS manufacturing process is enclosed and operates as a controlled release process on a continuous basis, up to 24 h/d, 335 d/y. In the processing plants, NOBS is formulated with other chemicals for ultimate use in granular and tablet detergents. The formulation process includes continuous production, dedicated equipment systems, where no releases occur during regular production. For equipment clean-up, hot water is used and disposed via the drain. Washed-away NOBS, if any, will be rapidly biodegraded and removed in the sewer and publicly owned treatment works connected to the processing plants. The Environmental fate of NOBS (surface water concentrations at the point of discharge for low flow, i.e., 7Q10) at the manufacturing and the production sites was modeled with E-FAST, a U.S. EPA screening model, using the “general population exposure from industrial releases” option. The assumptions used in this assessment approach included: 335 days of operation, 11,100 tonnes production, 0.15 % loss from equipment cleaning (e.g., wash down of the tower, scrubber water) and from spillage (U.S. EPA 1996), all the aqueous release goes to municipal waste water treatment before release to the environment. NOBS is manufactured at the Eastman Chemicals plant in Batesville, Arkansas (Table 4). 7Q10 represents the lowest flow for 7 consecutive days in a 10-year period and represents a high end to bounding estimate of dilution of the plant effluent.

Table 4: Details of NOBS manufacturing site (Batesville plant), according to E-FAST

FACILITY NAME: EASTMAN CHEMICAL CO		
FACILITY LOCATION: BATESVILLE AR72501		
RECEIVING WATER NAME: WHITE R		
REACH NUMBER: 11010004001	FACILITY ON REACH: No	DICHARGE TYPE: Indirect
NPDES PERMIT #: AR0035386	DATA SOURCE: FacSrch	

Integrating processed NOBS tonnage, loss rate from equipment cleaning and from spillage in plants, removal in waste water treatment plants, and number of processing days, the post-treatment discharges to the wastewater treatment plant at the Eastman Chemical plant was 2.5 kg NOBS/d, causing a predicted aquatic exposure concentration of 16 μg NOBS/l.

NOBS is incorporated into detergent formulas at the two following plants of the Procter & Gamble Company: Augusta - POTW NPDES # GA0020087 (Table 5) and Alexandria/Pineville - POTW NPDES # LA0033464 (Table 6). Fifty-five % of NOBS produced in the Eastman plant (i.e., 6,100 metric tons/y) is formulated in the Alexandria/Pineville plant, 45% (i.e., 5,000 metric tons/y) in the Augusta plant.

Table 5: Details of NOBS processing site (Augusta plant), according to E-FAST

FACILITY NAME: AUGUSTA WPCP		
FACILITY LOCATION: AUGUSTAGA30911		
RECEIVING WATER NAME: SAVANNAH R		
REACH NUMBER: 03060106045	FACILITY ON REACH: No	DICHARGE TYPE: Indirect
NPDES PERMIT #: GA0020087	DATA SOURCE: FacSrch	

Integrating processed NOBS tonnage, loss rate from equipment cleaning and from spillage in plants (0.15%), removal in waste waster treatment plants, and number of processing days (250 d/y), the post-treatment discharges to the wastewater treatment plant at the Augusta plant was 1.1 kg NOBS/d. The 7Q10 surface water concentration for the 10^{th} %tile low flow was 0.23 μg/l.

Table 6: Details of NOBS processing site (Pineville plant), according to E-FAST

FACILITY NAME: PINEVILLE CITY OF		
FACILITY LOCATION: PINEVILLLA71360		
RECEIVING STREAM NAME: RED R		
REACH NUMBER: 08040301020	FACILITY ON REACH: Yes	DISCHARGE TYPE: Direct
NPDES PERMIT #: LA0033464	DATA SOURCE: FacSrch	STATION ID: 07355500

Integrating processed NOBS tonnage, loss rate from equipment cleaning and from spillage in plants (0.15%), removal in waste waster treatment plants, and number of processing

days, the post-treatment discharge to wastewater treatment at the Pineville plant was 1.4 kg NOBS/d. The 7Q10 surface water concentration for the 10^{th} %tile low flow was 0.38 μg/l.

[2.3.3] Ecosystem Exposures Related to the Use and Disposal of Products Containing NOBS

NOBS enters the public domain in the form of household laundry products intended for disposal to sewer. The chemistry of NOBS during the wash (see degradation of NOBS in Appendix B) indicates that little, if any NOBS enters the environment under expected consumer use and disposal patterns. The detergent formulations containing NOBS are designed to help ensure rapid conversion in the wash water of NOBS to the peroxy compound. NOBS is short-lived (< 1% remaining after 3 minutes in wash) due to the instability of the peroxide (O-O) bond. To be effective, the bleach activator NOBS must be highly dispersed in water, undergo rapid perhydrolysis, and produce a fatty peracid bleach with some degree of surface activity. NOBS fulfills these requirements in the presence of bleach, e.g., sodium percarbonate or sodium perborate, in the laundry detergent product. Due to the presence of excess sodium percarbonate or sodium perborate in the product, the bleach activator molecule reacts quantitatively, within one minute, in an aqueous detergent solution to form primarily the peracid bleach (perhydrolysis). Phenol sulfonate is the other perhydrolysis product. The bleach activator molecule is designed so that the ester perhydrolysis, the preferred reaction, dominates over straightforward hydrolysis or diacyl peroxide formation.

[2.3.3.1] Reactions occurring in the wash solution

NOBS is stable in neutral (pH 7) solution but it readily degrades in acid (pH<6) and alkaline (pH>8) solutions, e.g., in the wash solution. A full discussion on NOBS perhydrolysis is reported in Appendix B. Perhydrolysis is the desired and favored reaction under wash conditions. Under the temperature and pH conditions created by the detergent formula in the wash solution, sodium perborate monohydrate releases hydrogen peroxide that reacts with NOBS to form the peroxy acid, pernonanoic acid, and at the same time releases phenol sulfonate. These reactions are completed within the first few minutes of the wash. The measured degradation of NOBS at 40°C in a 1% aqueous detergent solution was extremely fast: after 3 minutes, NOBS could no longer be detected. The reactions are somewhat slower in cold-water wash conditions but still completed within the wash cycle.

[2.3.3.2] Consumer Product Releases Influent Concentration

The concentration of NOBS in the influent of municipal wastewater treatment plants was estimated using E-FAST's Down-the-Drain scenario. We assumed per capita water use of 364 l/cap.day, a US population of 2.5×10^8 (EPA defaults), 99% degradation of 11,100 tonnes during the wash (see [2.3.3.3]), and no loss of NOBS in the sewage collection and conveyance system. Assuming a removal of 95% during waste water treatment, the 10th percentile low flow PEC was 0.04 ng NOBS/l and the 50th percentile low flow PEC was 0.003 ng NOBS/L.

[2.3.3.3] Summary of Predicted Surface Water Concentrations

Source	Predicted surface water concentrations
Manufacturing site (Batesville plant)	16 μg NOBS/l
Processing site (Augusta plant)	0.23 μg NOBS /l (7Q10, 10th %tile low flow)
Processing site (Pineville plant)	0.38 μg NOBS /l: (7Q10, 10th %tile low flow)
Consumer Product Use	0.003 ng NOBS/l(50th %) to 0.04 ng NOBS/l(10th %)

Environmental monitoring studies have not been performed, as modeled estimates suffice for this material.

[2.3.3.4] Other Sources of Ecological Exposure

There are no additional ecological exposure sources of NOBS (e.g. no non-chain of commerce or natural sources).

[2.4] Ecotoxicity

Three laboratory acute ecotoxicity studies are available for NOBS – for algae, daphnia, and fish (bluegill). The lowest acute toxicity value was 9.3 mg/l for the algae, *Selenastrum capricornutum* (E*b*C50). The US EPA assessment factor when acute toxicity test data are available for three different aquatic taxa is 100; therefore, the Predicted No Effect Concentration, PNEC, or Concentration of Concern, is 93 μg/l based on the available data. Although the fish and daphnia tests were conducted before GLP implementation in toxicity laboratories (1982), QSAR (ECOSAR, SRC Program) results confirmed that algae (predicted EC50 = 44 mg/l) were more sensitive to NOBS than fish (predicted LC50 = 560 mg/l) and daphnia (predicted LC50 = 3280 mg/l).

[2.4.1] Algal Growth Inhibition test

The acute E*b*C50 value was 9.3 mg/l for the algae, *Selenastrum capricornutum*. This test was supported by analytical confirmation of exposure concentrations. The geometric mean of the measured concentrations was calculated.

[2.4.2] Acute Fish test

The acute LC50 value was 32 mg/l for the fish, *Lepomis macrochirus*. The test was not supported by analytical confirmation of exposure concentrations. The dissolved oxygen levels dropped below 20% saturation after 48 h. It is at that time that mortality occurred. The reported fish mortality was mainly the result of stress due to low oxygen level. In addition, the stability test data (Robust summary 3.1.2) indicate NOBS to be stable in water, at neutral pH, for 96h.

[2.4.2] Acute Daphnia test

The acute LC50 value was greater than 1000 mg/l for *Daphnia magna*. The test was not supported by analytical confirmation of exposure concentrations.

[2.5] Environmental Screening Level Assessment

The risk to the aquatic environment is characterized by comparing the predicted exposure concentration (PEC) to the concentration of concern (CoC or PNEC). If the concentration in the surface water is less than the concentration of concern, then the potential for adverse effects is low. Integrating all the information currently available, NOBS PEC, at manufacturing and processing sites and in surface waters following consumer use, never exceeds the concentration of concern (93 μg/L). These assessments conservatively assume that neither hydrolysis nor perhydrolysis occurs following discharge at the manufacturing and processing sites and removal in wastewater treatment is 95% vs 99+% observed in studies. The table below presents the risk characterization ratio (PEC/PNEC) for each life-cycle stage of NOBS, i.e., from manufacturing to consumer use. The ratios below 1.0 confirm that the potential for environmental adverse effects from NOBS is very low. Degradation products from NOBS are also predicted to have low toxicity, be removed during wastewater treatment and not likely to persist in the environment.

Life-cycle stage	**PEC**	**PNEC**	**PEC/PNEC**
Manufacturing (Batesville)	16 μg NOBS/l	93 μg NOBS/l	0.17
Processing (Augusta)	0.23 μg NOBS /l		2.5×10^{-3}
Processing (Pineville)	0.38 μg NOBS /l		4.1×10^{-3}
Consumer Product Use	0.04 ng NOBS/l		4.3×10^{-7}

[2.6] References

Klimisch *et al.* (1997) Reg Tox Pharm 25: 1-5

Mackay D, DiGuardo A, Paterson S, Cowan CE (1996) Environ Tox Chem 15: 1627-1637

U.S. EPA (1996) Chemical Evaluation Branch (CEB) 8/16/1996. Generic Scenario: Surfactants in Industrial and Commercial Laundries. US EPA, Washington, DC

Draft guidance on developing robust summaries. http://www.epa.gov/chemrtk/robsumgd.htm

[3.0] Human Health Assessment

[3.1] Introduction

NOBS is a proprietary bleach activator exclusively used in granular and tablet laundry detergents. Each of the reports obtained was reviewed to determine adequacy according to EPA criteria and reliability per Klimisch *et al.* (1997). Robust summaries were prepared for each report with the highest Klimisch scores according to the guidelines recommended by the EPA (U.S. EPA, 1999) for each study type. Robust study summaries for SIDS endpoints, as well as several relevant endpoints beyond SIDS, with available and reliable (according to Klimisch criteria) data for NOBS are provided in Appendix A and are summarized in Table 7.

Table 7 Summary of SIDS Endpoints

Acute Mammalian Toxicity			
Acute Oral Toxicity	Rats	Oral gavage. 10 rats/group. Doses 5.10, 5.78, 6.46, and 7.14 g test material/kg body weight of 40%w/v suspension in distilled water.	Acute oral LD_{50} = 6.03 g/kg Klimisch 1
Acute Dermal Toxicity	Rabbits	24 hour exposure to 2 ml/kg of a 40% w/v aqueous solution on intact (3 rabbits) or abraded (3 rabbits) skin.	Acute dermal LD_{50} > 2.0 ml/kg (0.8 g/kg) Klimisch 1
Mutagenicity/Genotoxicity			
Ames Assay	Salmonella and E. coli strains	Plate incorporation method. Doses ranged from 50 to 7,000 µl/plate in the definitive study with and without S9 activation.	No evidence of mutagenicity. Klimisch 1
In vivo Chromosomal Aberration Assay	Rats	Acute and repeat dosing regimens used with doses ranging from 0.16 to 3.2 g/kg test material. Positive and negative controls also included.	The test compound has no clastogenic potential under the conditions of this test. Klimisch 1
In vivo Unscheduled DNA Synthesis Assay	Rats	Test material administered at 500, 1000, or 2000 mg/kg bw. Positive and negative controls included. Hepatocytes were harvested and evaluated.	Test article did not induce a significant increase in DNA synthesis. Klimisch 1
Developmental and Reproductive Toxicity			
Developmental Toxicity Study	Rats	Four dose groups 0, 500, 1000, or 1500 mg/kg/day by gavage on gestation days 6-15.	NOEL pup: 1500 mg/kg NOAEL dam: 500 mg/kg LOAEL dam: 1000 mg/kg Klimisch 1
Reproductive Toxicity Study	Rats	Comparable to one generation fertility study. Four dose groups 0, 100, 500, 1000 mg/kg/day by gavage.	NOEL repro: 1000 mg/kg NOAEL systemic: 100mg/kg Klimisch 1
Repeat Dose (Subchronic) Toxicity			
91 Day Oral Toxicity Study	Rats	Dietary levels of 0, 0.001, 0.01 and 0.1% (equivalent to 0, 10, 100, or 1000 mg/kg/day).	NOAEL: 0.11% in diet (approximately 1,100 mg/kg/day) Klimisch 1

Summary of Beyond SIDS Endpoints

Irritation/Corrosivity			
Eye Irritation	Rabbits	LVET method using neat material. No rinse (6 rabbits) and rinse (3 rabbits) conditions.	Slight to moderate irritation, some with corneal involvement, cleared by Day 7 (rinsed) or Day 14 (unrinsed). Klimisch 1
Eye Irritation	Rabbits	FHSA method using neat material without a rinse (3 rabbits), with a rinse (3 rabbits) or with a 10% w/v solution without a rinse (3 rabbits).	Mild to moderate irritation, some with corneal involvement. For the neat material, all irritation cleared within 4 days. For 10% solution, most effects reversible in 4 days and all effects reversible within 21 days Klimisch 1
Skin Irritation	Rabbits	Department of Transportation method. N=6 for 40% w/v suspension and N=6 for undiluted material	40% suspension was slight irritant and neat material was non-irritating. Klimisch 1
Skin Sensitization			
Skin Sensitization	Guinea Pigs	Modified Buehler. Induction dosed at 20% (occluded), challenge at 20%.	No evidence of skin sensitization under the conditions of this test. Klimisch 1
Skin Sensitization	Guinea Pigs	Modified Buehler. Induction dosed at 5.0% (occluded), challenge at 2.5%, rechallenge at 1%	Evidence of skin sensitization under the conditions of this test. Klimisch 1
Skin Sensitization	Guinea Pigs	Modified Buehler. Induction dosed at 10% (occluded), challenge at 0.5%.	No evidence of skin sensitization under the conditions of this test. Klimisch 1
Skin Sensitization	Mice	Local Lymph Node Assay 10%, 5%, 1%, 0.5%	No increase in lymph node cell proliferation and no evidence of skin sensitization under the conditions of this test. Klimisch 1
Repeat Dose (Subchronic) Toxicity			
28 Day Dermal Toxicity Study	Rabbits	0, 1.5% or 20% test material in water (2 ml/kg) dosed on abraded skin for 7 hours/day, 5 days/week for 4 weeks.	NOAEL: 2 ml/kg of 20% solution (0.4 g/kg) - no systemic toxicity. Effects limited to dose related dermal irritation at application test site. Klimisch 1

3.2 Hazard Assessment

The following toxicology data are provided in support of the use of NOBS as an ingredient in laundry detergents. A summary of each study is presented below. Additional information on these studies, including methods, is provided in Appendix A. Studies were conducted on NOBS (C9 AOBS), and in some cases on C8 AOBS, or C8/10 AOBS during the technical research and development of this bleach activator. Approximately 80% of the studies were conducted using the C9 AOBS material, 10% using the C8 AOBS material and 10% using a 50:50 blend of C8/C10 AOBS. These three test materials are identical in structure with the exception of the length of the alkyl chain. Based on published literature and unpublished P&G data, this difference in length of the carbon chain (C8, C9, and C10) is not expected to significantly effect the toxicity profile.

SIDS Endpoints

[3.2.1] Acute Oral Toxicity in Rats (C9 AOBS)

An acute oral LD_{50} toxicity study was conducted on NOBS. A single dose of the test material was administered as a 40% w/v aqueous solution to Sprague-Dawley rats by oral gavage. Test doses were 5.10, 5.78, 6.46 and 7.14 g test material/kg body weight. All animals (10 rats/group; 5 male, 5 female) were observed for mortality and clinical signs at 0.5, 1, 2, 3, and 4 hours after dosing and daily thereafter for 14 days.

The oral LD_{50} for male and female rats (combined Probit method) was calculated to be 6.03 g/kg body weight (95% confidence limits: 5.62 - 6.44 g / kg). All resulting mortality occurred within two days following administration of the test material. Clinical signs observed included diarrhea, abdominal gripping, hypoactivity and decreased respiratory rate. Generally, the signs and number of animals involved appeared to be dose related. All animals that died during the study had irritation or hemorrhaging of the stomach and intestine. All signs are consistent with surfactant related irritation of the GI tract. Necropsy results on surviving animals were unremarkable.

[3.2.2] Acute Dermal Toxicity in the Rabbit (C9 AOBS)

The acute percutaneous toxicity of NOBS was investigated in rabbits. A 40% w/v aqueous solution of the test material was applied to the skin (either abraded or intact) of the shoulder and rump of each rabbit at a dose of 2 ml/kg body weight and covered. Prior to treatment, hair was clipped from shoulder to rump. The skin of group I animals (3 rabbits) was left intact and the skin of group II animals (3 rabbits) was abraded with the clipper head. Test material was spread evenly over the clipped area and immediately covered with 8-ply gauze held in place by a dental dam covering the entire trunk. At the end of the 24-hour exposure period, dressings were removed and the treated area of the skin gently wiped to remove residual material.

Animals were observed for mortality and clinical signs at least once within 4 hours of dosing and daily thereafter for 14 days. If present, clinical signs and deaths were recorded at each observation time. The treated areas of skin were examined 30 minutes after removal of the test material, and then daily thereafter, for signs of dermal irritation. Dermal effects were assessed according to a pre-defined grading scale for erythema, edema and eschar. All animals were necropsied either upon death during the study or at the end of the 14 day observation period.

One animal from the abraded group died on Day 7 of non-treatment related causes (gastro-enteritis of unknown etiology). During the first 6 days following test material administration, dermal irritation ranged from slight to severe erythema, slight to moderate edema and slight atonia. Only slight erythema was observed beyond day 7. All animals had normal weight gain. Except for the local skin effects observed at the site of application, no treatment related gross effects were observed at necropsy. Based on the results of this study, the dermal LD_{50} of NOBS is greater than 2.0 ml/kg (greater than 0.8 g/kg).

[3.2.3] Mutagenicity (C9 AOBS) - Escherichia coli WP2 and WP2 uvrA Reverse Mutation Assay and Salmonella/Mammalian - Microsome Mutagenesis Assay (Ames Test)

The mutagenicity potential of NOBS was evaluated in the bacterial reverse mutation assay using the Escherichia coli WP2 and WP2 uvrA Reverse Mutation Assay and Salmonella/Mammalian-Microsome Mutagenesis Assay (Ames Test) using strains TA1535, TA100, TA1537, TA1538, and TA98. Test material concentrations ranged from 50-20,000 µl/plate in the preliminary toxicity dose range-finding studies and typically 50 to 7,000 µ l/plate in the definitive studies. Appropriate positive, solvent and sterility controls were used. Tester strain titers were determined. All dose levels of test material, solvent and positive controls were plated in triplicate. Following an approximate 48 hour incubation at 37°C, revertant colonies per plate were counted; for all replicate plating, mean revertant colonies were calculated. The results of the E. coli and salmonella/mammalian microsome reverse mutation assays indicate that under the condition of these studies, the test material did not show any evidence of mutagenic potential in any of the tester strains in the presence or absence of Arochlor-induced rat S9 liver microsomes.

Appropriate positive controls indicate a valid test. Both strains TA 100 (base-pair substitution) and TA 98 (frameshift) responded positively to 4-nitroquinoline-1-oxide and strain TA 100 also responded to N-methyl-N'-nitro-N-nitrosoguanidine. The activation systems were tested by positive responses to benzo[a]pyrene and 2-acetylaminofluorene.

[3.2.4] Structural Chromosomal Aberration (C8/10 AOBS)

The objective of this study was to evaluate the clastogenic potential of C8/10 AOBS as manifested by the production of chromosomal abnormalities such as deletions, exchanges, rings and breaks in bone marrow cells of treated rats.

Distilled water and methylmethane sulfonate served as the negative and positive control, respectively, in this study. Rats were dosed by gavage with 3.2 g/kg, 1.1 g/kg, or 0.32 g/kg in the acute dosing regimen phase and sacrificed at 6, 24, or 48 hours post dose while rats in the subchronic regimen received 1.6 g/kg, 0.5 g/kg, or 0.16 g/kg once a day for 5 days. Each dose group and sacrifice time consisted of 3 males and 3 females.

An i.p. injection of colchicine was given to inhibit mitosis approximately 2 hours prior to sacrifice. Bone marrow was collected, fixed, stained and analyzed for chromosomal abnormalities.

The appropriate positive and negative controls indicate a valid test. The results of this study indicate that C8/10 AOBS, administered orally over the dose range of 0.32 - 3.2 g/kg for the acute study and 0.16 - 1.6 g/kg for the subchronic study, did not induce a statistical increase in the number of chromosomal aberrations. Thus the compound has no clastogenic potential under the conditions of this test.

[3.2.5] *In vivo* Unscheduled DNA Synthesis (NOBS extrudate: 78% C9 AOBS)

The potential of NOBS to induce unscheduled DNA synthesis (UDS) in primary cultures of hepatocytes obtained from test article treated rats was assessed. In the *in vivo* UDS study, test and control articles were administered to male rats at a constant volume of 10 ml/kg body weight by a singe gavage injection. Sterile distilled water was selected as the vehicle for the test article. In the UDS assay, male rats were exposed to test article at 0.5, 1.0 and 2.0 g/kg body weight or to vehicle or positive control. No mortality was observed in any test article treated or vehicle control treated rats.

Treatment with NOBS did not induce a significant increase in the mean number of net nuclear grain counts (i.e., an increase of at least 5 counts over the negative control group) in hepatocytes isolated either 2 to 4 hours or 12 to 16 hours after dose administration. NOBS was concluded to be negative in the UDS test with mammalian liver cells *in vivo*.

[3.2.6] Teratology (C8/10 AOBS)

A study was conducted to determine the teratogenic potential of C8/10 AOBS in rats when dosed during organogenesis. Charles River CD rats received the test material once daily by gavage on gestation day 6 through 15, at one of the following doses: 0, 500, 1000, or 1,500 mg/kg/day. Each dose group consisted of 25 presumed pregnant female rats. Maternal body weights and food consumption were recorded on study day 0, 6, 9, 12, 16 and 20. Cesarean sections were performed on all surviving females on gestation day 20. Fetuses were individually weighed, sexed and examined for external malformations and variations. Approximately one half of the fetuses were placed in Bouin's solution for subsequent soft tissues examination using Wilson's sectioning technique. The remaining fetuses were prepared and stained with Alizarin Red S for skeletal examination.

No mortality was present over the course of the study in the control, 500, or 1000 mg/kg/day groups. Three rats dosed with 1500 mg/kg/day died on gestation 13 or 15. Necropsy observations of animals that died on study included reddened stomach mucosa and distended intestines. Clinical observations present in the mid and surviving high dose groups included respiratory rales and wet matted haircoat or material in the facial, ventral and/or anogenital regions. There were no meaningful differences in the gross necrospy of treated and control dams.

Oral administration of C8/10 AOBS from gestation day 6 through 15 resulted in a depression in maternal body weight change at all dosage levels during the first two measured intervals of treatment (days 6 to 9 and 9 to 12) and only in the high dose group during the last treatment interval (days 12 to 16). Mean food consumption was slightly decreased in the mid and high dose groups only during the treatment period.

There were no indications of a treatment related effect on fetal or embryonic growth or survival. Ovulation, implantation, intrauterine development, and embryogenesis were uniform in all study groups. Similarly, the occurrence of external, soft tissue and skeletal malformations and developmental variations was not different in the treated groups relative to the control group. When administered orally to pregnant Charles River CD rats, C8/10 AOBS did not induce a teratogenic effect at dosage levels of 500, 1000, or 1500 mg/kg/day.

The fetal NOEL was determined to be 1500 mg/kg/day and the maternal NOAEL was 500 mg/kg/day.

[3.2.7] Reproductive Toxicity (C9 AOBS)

A study was conducted to evaluate the potential for effects on reproduction and fertility in Sprague Dawley rats following repeated exposure to NOBS. Doses of 0, 100, 500, or 1000 mg/kg/day of NOBS in deionized water (dosing volume of 5ml/kg) was administered by oral gavage for 70 days prior to initiation of mating until termination which occurred on either gestation day 13 or lactation day 21. Each dose group consisted of 38 rats/sex. The F1 offspring were potentially exposed in utero and/or as neonates during lactation via maternal milk but did not directly receive the test article.

The estrous cycle was determined in females 10 days prior to mating until the end of the mating period. Body weights and food consumption were recorded weekly until copulation, on gestation days (GD) 0, 7, 13, and 20 and lactation days 0, 7, 14, and 21 for appropriate groups. Animals were observed daily for clinical signs of toxicity, changes in appearance, behavior and mortality.
In the uterine exam group (on GD13), the ovaries and uterine horns were examined for number of copora lutea, number of implantations, number and distribution of viable and nonviable fetuses, and early resorptions. For the dams that were allowed to deliver, litter size, number of still births, number of live births, and gross anomalies were determined. On postnatal day 4, litters were culled to 10 pups to achieve homogenous group size for evaluation of nursing, survival and body weight. Pups were weighed on postnatal day 0, 4, 7, 14, and 21. Tissues and organs from all F0 animals were macroscopically observed, with special attention to reproductive organs, and preserved in 10% neutral buffered formalin for potential microscopic evaluation.

There was no treatment related difference in the estrous cycle of female rats. Mortality occurred in 1, 1, 2, and 10 rats in the 0, 100, 500, and 1000 mg/kg day groups, respectively. Macroscopic observations noted in three females that died on study included gastric lesions with thickened tissue indicative of gastric irritation. Five males that died in the high dose group had pulmonary lesions suggestive of pneumonia. Test article was not directly implicated in the deaths. Clinical observations in the mid and high dose groups included excessive salivation and respiratory rales. There were no significant adverse effects on body weights or food consumption. The high dose males showed a slight yet consistent decrease in body weights (4% or less decrease) compared to control animals throughout the study. Uterine exam observations show no difference in the number of viable embryos, postimplantation loss, total implantations or number of corpora lutea. For the F0 delivery and F1 litter observations there was no test article effect observed on male or female fertility indices, copulatory indices, gestation length, mean number of live/dead pups on day 0, pup survival to weaning or pup body weight throughout lactation. There were no indications of a treatment related effect on fetal or embryonic growth or survival. Ovulation, implantation, intrauterine development, and embryogenesis were uniform in all study groups.

In conclusion, NOBS administered orally at dosage levels of 100, 500, or 1000 mg/kg/day did not result in adverse effects on fertility, parturition, neonatal viability, growth of the newborn or reproductive performance in rats.

[3.2.8] Subchronic (90 day) Feeding Study (C8 AOBS)

A subchronic feeding study was conducted to assess the potential for systemic toxicity after repeated exposure to C8 AOBS, a material closely related in structure to NOBS (two materials differ only in the length of the carbon chain, C8 vs C9). C8 AOBS was administered in diet daily for 13 weeks to 4 groups of Sprague Dawley rats (40 rats per group, 20 male and 20 female) at levels of 0, 0.001, 0.01 and 0.1% w/w, which equates to approximately 0, 10, 100, and 1000 mg/kg/day. Diets were prepared weekly and evaluated for homogeneity, stability, and dietary concentration of the test material. The concentrations were adjusted each week on the basis of a predicted mid week body weight and an estimate of food consumption for the week in question to provide a constant dose level in relation to body weight (mg/kg/day).

Animals were observed daily for overt signs of toxicity and mortality with detailed clinical examination at weekly intervals. Body weight and food consumption were recorded weekly throughout the study. Clinical chemistry evaluations were performed on blood and urine collected from 10 male and 10 female animals per group during week 12 and 13, respectively, and included hematology, blood chemistry and urinalysis. Opthalmoscopic examinations were performed on all animals in the control and high dose groups prior to the start of treatment, and during week 12.

Complete necropsies were performed on surviving animals at the end of the study. The following tissues were weighed and fixed: adrenals, heart, pituitary, brain, kidney, spleen, testes/ovaries, liver and thyroid. With the exception of the eyes which were fixed in Davidson's solutions, an extensive list of tissues were preserved in 10% neutral buffered formalin as noted in the protocol and Appendix A. All tissues from control and high dose animals, lung and liver tissue and gross lesions from low and intermediate dose groups were embedded in paraffin wax BP, sectioned at a nominal thickness of 5 microns and stained with haemtoxylin and eosin and evaluated by the pathologist.

Administration of C8 AOBS via the diet for 13 weeks did not result in any mortalities or induce any compound-related clinical signs of toxicity. There were no significant changes in body weights, food consumption, opthalmoscopy, clinical chemistry, urinalysis, absolute or relative organ weights or effects in the macroscopic and microscopic pathology.

Increases were observed between the control and high dose group males for neutrophils, lymphocytes, and BUN levels. In addition, creatinine and sodium were different for females. However, these changes were within the normal ranges observed in historical data compiled at the testing laboratory.

No toxicological significant treatment-related lesions were observed. The study established 0.11% in diet (approximately 1,110 mg/kg/day) as the no observed adverse effect level

(NOAEL) and AOBS was considered to be not systemically toxic to the rat up to a level of 1,100 mg/kg body weight/day.

Beyond SIDS Endpoints

[3.2.9] Primary Eye Irritation in the Rabbit (Low Volume Eye Irritation Procedure) (C9 AOBS)

NOBS was evaluated for the potential to cause eye irritation in the rabbit using the Low Volume Eye Test (LVET) procedure (ASTM #). Group I New Zealand White Rabbits received 0.01 ml of test material, placed directly on the cornea of one eye without rinsing. Group II rabbits received 0.01 ml of test material directly on the cornea followed by a rinsing procedure. Treatment groups consisted of 3 or 6 rabbits. Eyes were examined for corneal opacity, iritis and conjunctivitis and scored according to the methods of Draize (1959). The results for Group I (unrinsed) yielded a maximum average score of 33.7 (Day 2). Corneal involvement was observed in 6 of 6 animals. All effects observed were reversible (1 animal in 3 days, 1 in 4 days, 3 in 7 days and 1 in 14 days). The results for Group II (rinsed) yielded a maximum average score of 30 (Day 1). Corneal involvement was observed in 1 of 3 animals. Eyes of all animals returned to normal within 3-7 days (2 animals in 3 days and 1 in 7 days). The test substance caused slight to moderate irritation in all eyes that cleared by Day 7, except for one that cleared by Day 14.

[3.2.10] Primary Eye Irritation in the Rabbit (C9 AOBS)

To comply with European regulatory testing requirements, NOBS was tested in a Draize rabbit eye irritation study using New Zealand White Rabbits. Group I animals received 3 mg of test material placed in the conjuctival sac without rinsing. Group II animals received 3 mg of test material in the conjuctival sac followed by rinsing. Group III animals received 0.1 mL of a 10% w/v solution of the NOBS solution in the conjunctival sac without rinsing. Each groups consisted of 3 animals. Eyes were examined for corneal opacity, iritis and conjunctivitis and scored according to the methods of Draize (1959). The eyes were examined for irritation at specific time intervals, up to a maximum of 35 days, following treatment. The results for Group I yielded a maximum average score of 16.7 (Day 1). Corneal involvement was observed in 2 of 3 animals. All observed effects cleared within 4 days (2 animals in 3 days and 1 in 4 days). The results for Group II yielded a maximum average score of 5.3 (Day 1). Corneal involvement was observed in 0 of 3 animals. The mild conjunctival irritation was transient and cleared in 2 days in all subjects. The results for Group III yielded a maximum average score of 28.0 (Day 1). Corneal involvement was observed in 3 of 3 animals. All effects observed were moderate and reversible (2 animals in 4 days and 1 in 21 days). In summary, the test substance caused slight to moderate irritation in all eyes, which cleared by Day 4, except in the 10% w/v unrinsed group, which cleared by Day 21.

[3.2.11] Primary Dermal Irritation in Rabbits (C9 AOBS)

Two primary dermal irritation studies were conducted on NOBS using New Zealand White rabbits. In the first study, a volume of 0.5 mL of test material (40% w/v suspension of

NOBS in distilled water) was applied to a 1 x 1 inch gauze patch and occluded for 4 hours on intact unabraded skin. In the second study, 0.5 g of test material, slightly moistened with 0.9% saline was applied to a 1 x 1 inch gauze patch and occluded for 4 hours on intact, unabraded skin. Each study consisted of 6 rabbits. After 4 hours of exposure, the patches were removed from animals in both studies and the application sites were graded for irritation and corrosion.

The skin in the area of the application site was graded again at the end of 48 hours (44 hours after first reading). A standardized grading scale, ranging from 0 to 4, was used to score erythema, edema, and other skin effects if present including eschar, ulceration and necrosis. The average dermal irritation scores for animals in the first study at 4 hours were 0.54 and 0 for erythema and edema, respectively; whereas, at 48 hours the scores were 1.3 and 0 for erythema and edema, respectively. The calculated primary irritation index (PII, can range from 0-8 with 0 being a non-irritant) was 0.9, which classifies NOBS as a slight irritant. The average dermal irritation scores for animals in the second study at 4 and 48 hours were 0 for erythema and edema. Under the test conditions in the second study, undiluted NOBS was non-irritating and non-corrosive.

Dermal Sensitization

Based on the weight of evidence from all available data, which evaluated the potential of NOBS and laundry products containing NOBS to cause skin sensitization, it is concluded that NOBS can safely be used in products at levels up to at least 6%. Many of the studies included in the weight of evidence assessment are briefly summarized below. The types of studies included are non-clinical animal studies in guinea pigs and mice, clinical studies which use a variety of test methods including human repeat insult patch tests (HRIPTs), extended home use studies, provocative use studies, hand immersion tests, and T-shirt wear tests.

In 1986, P&G's approach to assess skin safety for laundry products was reviewed, critiqued and approved by a group of 16 world-renowned dermatology experts. In addition, this same group also reviewed and supported the finding that the use of NOBS in product at up to 6% was without risk of skin sensitization as had been demonstrated in non-clinical and clinical studies. As part of regulatory filings, authoritative bodies have concurred with the same conclusion. An overview of the numerous clinical studies conducted on the NOBS material alone as well as in product at up to 6% follows the non-clinical data summary.

Non-clinical animal studies

Four studies are summarized below which investigated the potential of NOBS alone to cause skin sensitization under exaggerated concentrations and conditions. Several studies were conducted using the NOBS material as manufacturing processes and starting materials varied during the research phase of the development process. Results from the non-clinical studies suggest NOBS as a raw material has the potential to act as a weak skin sensitizer in the guinea pig, although these effects are not consistently replicated in all animal studies. One reason for the inconsistent results may be related to the concentration of the active NOBS material. The test material in the first two guinea pig studies contained >95%

NOBS whereas the last two studies were conducted on material in the extrudate form used in laundry detergent products—78% C9 AOBS. The study to evaluate sensitization potential in mice resulted in no evidence of skin allergenicity under the conditions of the Local Lymph Node Assay (LLNA).

[3.2.12] Dermal Sensitization in the Guinea Pig (C9 AOBS)

The potential for delayed contact hypersensitivity reactions to NOBS was evaluated in Hartley albino guinea pigs (10 control animals; 20 test animals). Test material was applied as a 20% solution of NOBS (w/v) in water during induction. The induction concentration was selected based on the minimally irritating dose from skin irritation information for a similar compound. A screening study was conducted to determine the highest non-irritating concentration for challenge. Based on the results, a 20% (w/v, 0.4 ml) aqueous solution was used as the challenge concentration. At 24 and 48 hours post challenge, depilated animals were scored for erythema using a 0-3 scale (0= no reaction, ± = slight patchy erythema, 1= slight, but confluent or moderate, patchy erythema, 2= moderate erythema, 3= severe erythema with or without edema). No evidence of sensitization was observed in guinea pigs exposed under the conditions of this study.

[3.2.13] Dermal Sensitization in the Guinea Pig (C9 AOBS)

A second study was conducted using the modified Buehler protocol. The potential for delayed contact hypersensitivity reactions to NOBS was evaluated in Hartley albino guinea pigs (10 control animals; 20 test animals). Test material was applied as a 5% NOBS aqueous solution (w/v, 0.4 ml) under occlusive patch for six hours once per week during induction. The induction concentration was selected based on the irritating dose from the induction range finding study. A separate range finding study was conducted to determine the concentration for challenge. Based on the results, a 2.5% (w/v, 0.3 ml) solution was used as the challenge concentration. Test animals were rechallenged and a naïve control group was dosed with a 1% solution of test material in distilled water for six hours. Skin was graded at 24 and 44 hours after rechallenge, followed by depilation, and another skin grade at 48 hours.

The test sites were graded for skin responses, including erythema and edema, using a standardized scoring scale at 24 and 48 hours following chamber application at induction. During challenge, the test sites were graded through hair at 19 hours and then following depilation at 24 and 48 hours after patch removal. Irritation was noted during induction. At the 48 hr scoring interval following challenge, a dermal score of 1 was noted in 6/20 and 0/10 test and challenge control animals, respectively. All other scores ranged from 0 to ± in all other test and control animals. During the rechallenge phase, 2/20 test animals presented with a score greater than +/- at 24 and/or 48 hours after rechallenge. The two animals that responded positively during rechallenge also reacted in the challenge phase of the study. Under the conditions of this study, the data indicate a contact sensitization response occurred in some of the test animals at the concentrations tested.

[3.2.14] Dermal Sensitization in the Guinea Pig (NOBS extrudate: 78% C9 AOBS)

A recent study was conducted using the modified Buehler protocol and a sample of NOBS as used in end use product. The potential for delayed contact hypersensitivity reactions to NOBS was evaluated in Hartley albino guinea pigs (10 control animals; 20 test animals). Test material was applied as a 10% solution (w/v, 0.3 ml) under occlusive patch for six hours once per week during induction. The induction concentration was selected based on the minimally irritating dose from the induction range finding animals. A range finding study was conducted to determine the highest non-irritating concentration for challenge. Based on the results, a 0.5% (w/v, 0.3 ml) solution was used as the challenge concentration.

The test sites were graded for skin responses, including erythema and edema, using a standardized scoring scale at 24 and 48 hours following chamber application at induction. During challenge, the test sites were graded through hair at 19 hours and then following depilation at 24 and 48 hours after patch removal. Irritation was noted during induction. At the 24 and 48 hr scoring interval, dermal score of 1 was noted in 1/20 and 0/10 test and naïve control animals, respectively. All other scores ranged from 0 to ± in all other test and control animals. Under the conditions of this study, the test material is not considered to be positive for skin sensitization based on EPA and OECD guidelines.

[3.2.15] Dermal Sensitization in the Mouse (NOBS extrudate: 78% C9 AOBS)

A study evaluated the potential of NOBS to be a skin allergen in mice by using the Local Lymph Node Assay (LLNA). The study consisted of 4 dose groups, a vehicle control group (reverse osmosis water) and a naïve control group, each with 5 mice/group. For each treatment group, five mice were treated daily for three consecutive days by direct epicutaneous application of 25 µl of test article to each ear. In addition a vehicle control (reverse osmosis water) and a naïve control (no treatment) were evaluated. Approximately 71 hours after final test application, mice were injected i.v. in the tail vein with tritiated thymidine to label proliferating cells.

Mice were observed immediately prior to and approximately 2-4 hours after dosing for any significant alterations in appearance of the application site. Mice were observed twice daily for general health and mortality. Five hours after injection, lymph nodes were harvested and single cell suspensions prepared and quantitated by liquid scintillation spectrometry.

All animals appeared normal throughout the study. Body weight gain was noted for all treatment animals during the day -1 and day 6 interval. The stimulation indices of lymph nodes were calculated for each treatment group compared to controls. The groups treated with 10%, 5.0%, 1.0% and 0.5% demonstrated stimulation indices of 0.5, 0.6, 0.9, and 0.7, respectively. A stimulation index of 3.0 (three fold increase over controls) would be considered a positive immunological response for sensitization.

Treatment with the test article did not result in an increase in lymph node proliferation compared to controls demonstrating the test material is not a dermal contact allergen under the conditions of this test.

Summary of the human clinical data

The data summarized below show that laundry detergent product containing up to 6% of NOBS is not expected to present a risk of skin sensitization to consumers.

As noted above, in some animal studies NOBS has the potential to cause contact sensitization in animals when tested under exaggerated concentration and exposure conditions. Human sensitization studies on NOBS alone in over 2,499 volunteers who gave informed consent resulted in 1 weak positive response. That person was able to use detergent product containing up to 6% of NOBS for 14 months at home without skin problems and was found negative in a subsequent diagnostic patch test. In addition, none of the volunteers (>2,000) who participated in clinical tests with NOBS-containing product developed contact sensitization. These tests mimicked under exaggerated conditions the typical use of laundry detergent in the US and included product usage in automatic machines but also product usage in laundry hand-wash lasting at least 10 minutes and pre-treatment of fabrics.

Additional clinical studies included several different methodologies and test designs. The studies below all support the conclusion that NOBS can be safely used in laundry products under use and exaggerated use condition without the risk of skin sensitization. The type of studies include:

- Extended home use tests (n=268 volunteers for 3 months with patch test before and after test)
- Extended laundry pretreatment tests (n=87 volunteers used 50% paste of detergent containing 6% NOBS for 8 weeks with patch test before and after test)
- Extended home use pretreatment (n=117 volunteers used 60% paste of detergent containing NOBS for 12 weeks with patch test at end of study)
- Provocative use test for 14 months with patch testing at 7 and 14 months
- Hand immersion test (n=26 volunteers soaked hands for four consecutive days, include diagnostic patch test before and after study)
- T-shirt wear test (n=130 male volunteers with sensitive skin, in hot humid climate wore T shirts laundered in detergent containing NOBS)

[3.2.16] Repeated Dose Dermal Toxicity in Rabbits (C8/10 AOBS)

The purpose of the study was to assess the percutaneous and systemic toxicity of test article when it is repeatedly applied to abraded skin of New Zealand white rabbits over a period of 28 days. The test material for this study was 50% sodium octanoyloxybenzene sulfonate (C8 AOBS) and 50% decanoyloxybenzene sulfonate (C10 AOBS), which differs from NOBS only in the length of the carbon chain (C8 and C10 vs C9).

For 4 weeks, ten animals per group each were exposed for 7 hours/day, 5 days/ week on abraded skin to 2 ml/kg of water, 1.5% or 20% C8/10 AOBS. Each day of dosing the skin was graded and observations for clinical signs of toxicity were made. Body weights were measured once per week and tissues were taken at the end of the study for microscopic evaluations. The weights of the liver and kidneys were measured and the hemogram for each animal was determined.

No animals died and there were no test article related overt signs of toxicity. There were no differences between the controls and test groups with regard to body weight, weight gain, hematology values and absolute or relative organ weight. The most common symptoms reported were soft stools and diarrhea. These were seen across all groups and both sexes and did not appear to be test article related. There were no test article related gross or microscopic changes observed in any tissues examined except skin. Any differences in hematology endpoints were within the normal clinical limits.

Skin responses, both gross and microscopic, increased with the concentration of test article. Slight erythema and desquamation were observed in the 1.5% group. Exposure to 20% C8/10 AOBS caused slight erythema, edema and desquamation and slight to moderate atonia and fissuring. The microscopic evaluation of the skin from this group revealed dermal effects that included inflammation, parakeratosis, acanthosis, hyperkeratosis, and vesiculation.

The application of test material at levels up to 20% to the abraded skin of rabbits did not cause any detectable systemic toxicity. The effects of C8/10 AOBS in rabbits appears to be limited to dermal irritation and microscopic effects localized to the test application site when applied to the skin in a concentration up to 20% w/v and dosed five days per week for four weeks. The degree of irritation appears to be dose-related.

[3.2.17] Absorption, distribution, metabolism, and excretion (ADME) study (C9 AOBS)

Oral and dermal absorption, distribution, excretion (ADE) studies have been performed in rats using uniformly ring-labelled C9 AOBS (approx. 11 mg/kg). The radiochemical purity of the test material was 97%. The dermal ADME study showed there was no significant absorption by this route of exposure. Less than 1% was absorbed with 0.56 $\pm$ 0.18% eliminated from urine, < 0.02% via CO2, and < 0.16% via faeces after 72 hours. Recovery from the skin application site and the cage wash was 99.1 $\pm$ 1.0% and 0.14 $\pm$ 0.06%, respectively. Total recovery was 101.9 $\pm$ 0.7%.

NOBS was rapidly absorbed and eliminated in the oral (gavage) ADME study. Essentially all of the oral dose was eliminated in 72 hours; 80.2 $\pm$ 8% via urine, 1.6 $\pm$ 0.1% via faeces, and < 0.22% via CO2, and 19.7 $\pm$ 6.1% via the cage wash. At 72 hours after dosing, there was no concentration of the 14C-labelled material in any of the tissues examined including reproductive tissues. Bile duct canulation showed enterohepatic circulation did not occur. Total recovery was 101.8 $\pm$ 3.3%. HPLC analysis of the urine showed that no parent compound was excreted. Approximately 99% of the radioactivity in the urine represented a single metabolite consistent in HPLC retention time with hydroxybenzene sulphonate (phenol sulphonate).

These ADME data indicate that NOBS is very poorly absorbed upon dermal exposure (the anticipated major route of potential exposure) and highly absorbed following oral exposure. Absorbed material appears to be rapidly metabolised (via cleavage of the ester linkage) with excretion of the phenol sulphonate moiety and assumed normal catabolism of the fatty

acid moiety via the established odd-chain fatty acid pattern (AL Lehninger, Biochemistry, 2nd edition, 1975, chapter 20, p.555).

Human Exposure Assessment

Table 8 Consumer Exposure for NOBS used in a Laundry Detergent

Use or Exposure	Estimated Exposure Level	Relevant No Effect Level
Dermal Exposure	4.2×10^{-4} g/kg/day	0.4 g/kg/day
Oral Exposure Drinking Water	3.9×10^{-8} mg/kg/day	1.11 g/kg/day

[3.3] Worker Exposure

There is potential for occupational exposure to this material by workers who either produce the raw material or formulate the laundry detergent containing the material. The potential routes of exposure that are most relevant during manufacture of NOBS and formulation of laundry detergents containing NOBS are dermal and inhalation exposure.

Manufacturing Facility

For workers, exposure to NOBS during the production or transportation process is limited due to process design, industrial hygiene standards and personal protective equipment that are standardly utilitized in production facilities. Employee exposure is minimized through engineering controls, a closed system operation and good industrial hygiene practices to ensure exposure is below an Occupational Exposure Guideline (OEG) of 0.1 mg/m^3. The substance is produced and shipped as a low vapor pressure, non-respirable extrudate preparation with particle size of 500-1000 microns, which further limits the potential for exposure. Dermal exposure is limited by use of personal protective equipment, goggles and impervious gloves worn in direct handling of NOBS and in case of spills, maintenance, cleaning and process intervention.

Formulation Facility

The potential for worker exposure during the manufacture of detergents containing NOBS is minimized through engineering controls, a closed system operation, administrative procedure and personal protective equipment. Periodic monitoring indicates concentrations well below the OEG. All systems which use NOBS are enclosed, maintained under negative pressure, and have sufficient local exhaust ventilation airflow. Good occupational hygiene practices are defined for all operations such as sampling and systems maintenance. Operators involved in these procedures are trained on the potential hazards of the materials, controls and safe practices. Dermal exposure is limited by personal protective equipment, goggles and impervious gloves worn with direct handling of NOBS and in case of spills, maintenance, cleaning and process intervention. A behavior observation and safety sampling system is in place as part of standard operating procedures to reinforce compliance with safe practices. In case of accidental spillage, high efficiency filter portable vacuum cleaners are used routinely for clean up. Processing experience with a variety of

ingredients in the manufacturing of laundry detergents show that the above combination of engineering controls and work practices is effective in minimizing worker exposure.

[3.4] Consumer Residential Exposure

Residential exposure to NOBS from consumer use is expected to be limited based on the use pattern for the product and the chemistry of NOBS. There will be no consistent or significant consumer exposure to unreacted NOBS and the only possible residential exposure to NOBS is through use of laundry products containing this material. Consumer exposure with the tablet form of the product is expected to be the same as or less than with the granular form. In the vast majority of cases, laundry detergent containing NOBS is used in conjunction with an automatic washing machine, which greatly limits potential consumer exposure to unreacted NOBS. The potential routes of consumer exposure are discussed below and are followed by calculations to estimate the most relevant exposures. Consumer monitoring studies have not been performed, as modeled estimates suffice for this material.

[3.4.1] Dermal - The potential sources for dermal exposure when using laundry detergents containing NOBS are 1) during hand laundering, 2) during pretreatment of fabrics with a paste made from detergent, or 3) from skin contact during transfer of the product from the package to the washing machine. The potential for dermal exposure from scooping the product from the package is infrequent and negligible relative to the hand laundering or fabric pretreat scenarios. The transfer of product from the box to the washing machine is completed using a scoop; therefore, the potential for that dermal exposure to NOBS is negligible and of very short duration. Based on insignificant exposure from the latter scenario, exposure calculations are not included. There is no NOBS exposure from residual detergent that may remain on washed fabrics due to the rapid chemical reaction and complete perhydrolysis of this material in the wash water.

Under the typical conditions of the wash, NOBS is converted extremely rapidly to the peroxy compound pernonanoic acid within 3 minutes. Based on the chemistry and timing of this reaction, the following exposure calculations are conservative. In addition, these exposures are of very short duration, in the range of 5-10 minutes per task, which is not considered in the calculations. A summary of the dermal exposure estimates is included in the table below and in more detail in the following section.

Table 9 Consumer Dermal Exposure

Dermal	Exposure	Resulting Dose*
a. Hand Laundry	7.5×10^{-6} g/kg/day	7.5×10^{-8} g/kg/day
b. Fabric Pretreatment	4.1×10^{-4} g/kg/day	4.1×10^{-6} g/kg/day
TOTAL DERMAL EXPOSURE	**4.2×10^{-4} g/kg/day**	**4.2×10^{-6} g/kg/day**

* The resulting dose takes into account the estimated dermal absorption of NOBS which is <1%.

Dermal-Hand Laundering Fabric

Exposure during hand laundry is given by the following equation:

$$\text{Exposure}_{(\text{hand laun.})} = \frac{(\text{tasks/day}) \times (\text{vol. of sol'n on skin}) \times (\text{conc. of HPV substance})}{\text{Body weight}}$$

The dose resulting from this exposure is:

$$\text{Resulting Dose}_{(\text{hand laun.})} = \text{Exposure}_{(\text{hand laun.})} \times \% \text{ absorption}$$

Assumptions:

1. Product is used an average of 0.38 times/day for hand laundry. [1] Therefore,
   (tasks/day) = 0.38
2. The thickness of the wash solution on the skin is 0.0024 cm. [2]
3. The surface area of the hands and forearms is 1900 cm^2. [3]
4. Therefore, the volume of wash solution on the skin =
   (vol. of sol'n on skin) = (0.0024 cm) × (1900 cm^2) = 4.6 cm^3
5. Finished consumer product will contain approximately 6.0% HPV substance.
6. Use concentration of finished consumer product is 0.5%, or 5 mg/ml of wash solution[4]
7. Therefore, concentration of HPV substance in wash solution =
   (conc. of HPV substance) = 5 mg/ml × 0.06% = .30 mg/ml or 3.0×10^{-4} g/cm^3
8. Average adult body weight is 70 kg.

Therefore, exposure during hand laundry is:

$$\text{Exposure}_{(\text{hand laun.})} = \frac{(\text{tasks/day}) \times (\text{vol. of sol'n on skin}) \times (\text{conc. of HPV substance})}{\text{Body weight}}$$

$$= \frac{(0.38 \text{ per day}) \times (4.6 \text{ cm}^3) \times (3.0 \times 10^{-4} \text{ g/cm}^3)}{70 \text{ kg}} = \mathbf{7.5 \times 10^{-6}\ g/kg/day}$$

Percutaneous absorption (% absorption) of substances related to the HPV is less than 1% per ADME study. Therefore the dose resulting from this exposure is:

$$\text{Resulting Dose}_{(\text{hand laun.})} = \text{Exposure}_{(\text{hand laun.})} \times \% \text{ absorption}$$

$$= 7.5 \times 10^{-6} \text{ g/kg/day} \times 1\% = \mathbf{7.5 \times 10^{-8}\ g/kg/day}$$

[1] Unpublished P&G data, multiple studies

[2] Westat, Inc. and Battelle Columbus Laboratories Report to EPA (1985). Subcontract #A-314DS (8149)-270 and contract #68-01-6721. National Household Cleaning and Painting Surveys.

[3] EPA Exposure Factors Handbook, August 1997. Table 6-4, page 6-14.

[4] Unpublished P&G data, HPT #1456-56.

Dermal-Fabric Pretreatment

Exposure during fabric pretreatment is given by the following equation:

$$\text{Exposure}_{(\text{pretreat.})} = \frac{(\text{tasks/day}) \times (\text{vol. of sol'n on skin}) \times (\text{conc. of HPV substance})}{\text{Body weight}}$$

The dose resulting from this exposure is:

$$\text{Resulting Dose}_{(\text{pretreat.})} = \text{Exposure}_{(\text{pretreat.})} \times \% \text{ absorption}$$

Assumptions:

1. Product is used an average of 1 time/day for fabric pretreatment. [5] Therefore, (tasks/day) = 1
2. The thickness of the wash solution on the skin is 0.0024 cm. [6]
3. Fabric pretreatment is done with a skin surface area approximately half the surface area of the palms, or one quarter of the surface area of the hands [7], or $0.08\ m^2 \times 0.25 = 0.02\ m^2$ or $200\ cm^2$.
4. Therefore, the volume of wash solution on the skin = (vol. of sol'n on skin) = $(0.0024\ cm) \times (200\ cm^2) = 0.48\ cm^3$
5. Finished consumer product will contain approximately 6.0% of HPV substance.
6. Finished consumer product is used undiluted for fabric pretreatment (or 1000 mg/ml or $1\ g/cm^3$).
7. Therefore, concentration of HPV substance used for pretreatment = (conc. of HPV substance) = $6.0\% \times 1\ g/cm^3 = 0.06\ g/cm^3$
8. Average adult body weight is 70 kg.

Therefore, exposure during fabric pretreatment is:

$$\text{Exposure}_{(\text{pretreat.})} = \frac{(\text{tasks/day}) \times (\text{vol. of sol'n on skin}) \times (\text{conc. of HPV substance})}{\text{Body weight}}$$

$$= \frac{(1 \text{ per day}) \times (0.48\ cm^3) \times 0.06\ g/cm^3}{70\ kg} = \mathbf{4.1 \times 10^{-4}\ g/kg/day}$$

9. Percutaneous absorption of HPV substance is less than 1% per ADME study. Therefore the dose resulting from this exposure is:

$$\text{Resulting Dose}_{(\text{pretreat.})} = \text{Exposure}_{(\text{pretreat.})} \times \% \text{ absorption}$$

$$= 4.1 \times 10^{-4}\ g/kg/day \times 1\% = \mathbf{4.1 \times 10^{-6}\ g/kg/day}$$

[5] Unpublished P&G data, - MRD 91D520, NHATS.

[6] Westat, Inc. and Battelle Columbus Laboratories Report to EPA (1985). Subcontract #A-314DS (8149)-270 and contract #68-01-6721. National Household Cleaning and Painting Surveys.

[7] EPA Exposure Factors Handbook, August 1997. Table 6-4, page 6-14.

[3.4.2] Oral - There is no anticipated oral exposure under use conditions. Due to the chemistry of NOBS, the potential level in drinking water is negligible to nil. The EFAST model was used to conservatively estimate the concentration of HPV substance in drinking water (assumes perhydrolysis is not complete, that some NOBS remains and none is removed in drinking water treatment facilities). The results of this model indicate the high end (10%ile) drinking water results to be 3.9×10^{-8} mg/kg/day.

The other potential for oral exposure would only occur following accidental ingestion of the product, which would be a one time or infrequent acute exposure. Based on information collected from the Procter & Gamble consumer telephone service, Poison Control Centers and national emergency rooms, when accidental swallowing does occur there are usually no symptoms reported. Occasionally, when symptoms do occur they include nausea, vomiting, or diarrhea, which are mild and transient in nature. These symptoms are not specific to NOBS since they would arise from accidental exposures to product containing NOBS and are symptoms consistent with ingestion of other laundry products.

[3.4.3] Inhalation - Consumer inhalation exposure during use is limited by a number of factors: the low vapor pressure of NOBS, its production in extrudate form, and the overall design of the laundry product as a non-friable, dense granular material. Thus, there is very little dust involved in transferring the product from the package to the washing machine so the potential for inhalation exposure from this action is negligible.

[3.4.4] Aggregate Exposure - As NOBS is a proprietary material, there are no other sources of consumer exposure to this material. As discussed above, there is minimal consumer exposure to NOBS when used in laundry detergents. Even when considering aggregate exposure, the amount of potential total exposure remains low.

[3.4.5] Comparison to E-Fast - To provide a basis for understanding how the results of an assessment conducted by the US EPA for consumer exposure might differ from the P&G assessment, the **E-FAST** model (Exposure and Fate Assessment Screening Tool) was used to evaluate the consumer exposure to NOBS. E-FAST was developed by Versar, Inc. for U.S. EPA's Office of Pollution Prevention, Economics, Exposure and Technology Division. E-FAST provides screening level estimates of concentrations of chemicals released to air, surface water, landfills, and from consumer products and can estimate potential dermal, inhalation and ingestion rates resulting from these releases. Modeled estimates of concentrations and doses are designed to provide high end to bounding estimates of exposure for use in screening level assessments. Information about E-FAST is available via OPPT's Exposure Assessment Tools and Models Web site: www.epa.gov/opptintr/exposure.

E-FAST can be used for a number of consumer product scenarios. NOBS is used in P&G granular laundry detergents, however, E-FAST's Liquid Laundry Detergent scenario was available and used. As discussed in the previous section, the most likely scenarios for consumer exposure to NOBS are skin contact during hand laundering and during use of a concentrated paste for pretreatment of fabric. The exposure estimates from completing the

E-FAST calculations for the use of NOBS at 6% in laundry products are shown in Table 10 and compared to estimates derived from using exposure calculations described earlier and routinely used by Procter & Gamble. A more complete description of this comparison can be found in Appendix C.

Table 10 Comparison of External Exposure Calculated by E-FAST and P&G

Scenario	E-FAST	P&G
Hand Laundry	1.3×10^{-6} g/kg/day	7.5×10^{-6} g/kg/day
Pretreatment	6.6×10^{-4} g/kg/day	4.1×10^{-4} g/kg/day

The above estimates conservatively assume 100% absorption. When there is evidence to support less than 100% dermal penetration the resulting internal dose may be determined by multiplying the external exposure by a dermal penetration fraction. The ADME study found that NOBS was poorly absorbed by the dermal route (less than 1%). E-FAST does not allow for the use of a dermal absorption fraction. Therefore, this needs to be calculated by hand from the E-FAST results, and is shown in Table 11.

Table 11 Comparison of Internal Doses Calculated by E-FAST and P&G

Scenario	E-FAST	P&G
Hand Laundry	1.3×10^{-8} g/kg/day	7.5×10^{-8} g/kg/day
Pretreatment	6.6×10^{-6} g/kg/day	4.1×10^{-6} g/kg/day

Conclusion: The consumer exposure estimates from the E-FAST runs are comparable in magnitude to those estimates derived from typical calculations developed by P&G. Both methods arrived at an external dermal exposure without consideration of dermal penetration of less than 0.01 mg/kg/day from hand laundering of fabrics and less than 1 mg/kg/day for pretreatment for a 6% NOBS granular laundry detergent. The resulting internal dose is less than 0.0001 mg/kg/day from hand laundering and less than 0.01 mg/kg/day for pretreatment. Using either method, the exposure estimates demonstrate very low potential for consumer exposure to NOBS from use of a granular laundry detergent.

[3.5] Human Health Screening Level Assessment

The available data summarized in this document demonstrate that NOBS has a favorable safety profile for use in consumer laundry detergents. The risk to human health is characterized by comparing the estimated exposure to the No Observable Effect Level (NOEL) from animal studies. The amount by which the NOEL exceeds the estimated exposure is referred to as the margin of exposure and this should be sufficiently large to account for several sources of uncertainty and variability in extrapolating data from animal studies to man. Based on the data presented, no adverse effects for humans are expected via any relevant exposure route. The aggregate dermal exposure from hand laundering and

pretreatment of fabrics results in an estimated exposure of 4.2×10^{-4} g/kg/day. In comparing this conservative estimate to the results from the dermal subchronic study where the no effect level (NOEL) is greater than 0.4 g/kg/day, the margin of exposure is acceptable. Even at the highest dose tested in this study (2 ml/kg of a 20% test material solution) there were no systemic effects. The only effect noted was irritation at the site of application, which was dose related and limited the amount that could be repeatedly administered. Studies evaluating the dermal absorption of NOBS showed this material is very poorly absorbed through the skin—less than 1%. For potential oral exposure, if one assumes conservatively that perhydrolysis does not occur, that NOBS would be present in drinking water and not removed in drinking water treatment facilities, the calculated exposure using EFAST would be 3.9×10^{-8} mg/kg/day. The NOEL in an oral dietary study was 1.1 g/kg/day. Comparing the estimated oral exposure to the oral NOEL results in a margin of exposure of many orders of magnitude, even after accommodating inter- and intraspecies variation.

[3.6] References

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